## REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely correct typographical errors in the specification, and insert the paper copy of the Sequence Listing and sequence identifiers in the specification. No new matter has been added.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: August 23, 2001

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**"Version With Markings to Show Changes Made"**

In the specification:

Paragraph beginning at page 5, line 26, has been amended as follows:

Some examples of preferred peptides include tetraamino acid sequences such as Ser-Lys-Leu-Gln (SEQ ID NO:1), Ile-Ser-Tyr-Gln (SEQ ID NO:2), and Lys-Ser-Lys-Gln (SEQ ID NO:3). Some examples of preferred pentaamino acid sequences are Ser-Ser-Lys-Leu-Gln (SEQ ID NO:4), Lys-Ile-Ser-Tyr-Gln (SEQ ID NO:5), and Thr-Lys-Ser-Lys-Gln (SEQ ID NO:6). Some examples of preferred hexaamino acid sequences are His-Ser-Ser-Lys-Leu-Gln (SEQ ID NO:7), Asn-Lys-Ile-Ser-Tyr-Gln (SEQ ID NO:8), and Ala-Thr-Lys-Ser-Lys-Gln (SEQ ID NO:9). Some examples of preferred heptaamino acid sequences are Glu-His-Ser-Ser-Lys-Leu-Gln (SEQ ID NO:10), Gln-Asn-Lys-Ile-Ser-Tyr-Gln (SEQ ID NO:11), and Glu-Asn-Lys-Ile-Ser-Tyr-Gln (SEQ ID NO:12). As noted, further amino acids can comprise $X_{-1}$.

Paragraph beginning at page 23, line 14, has been amended as follows:

Each assay contains 200 picomoles of the particular protease and 0.2 mM concentration of the particular substrate (*i.e.*, 40,000 picomoles/200 μL of assay volume). The amino acid sequences expressed in one letter amino acid code represent the following amino acid sequences in the three letter amino acid code: EHSSKLQ (Glu-His-Ser-Ser-Lys-Leu-Gln; SEQ ID NO:10), QNKISYQ (Gln-Asn-Lys-Ile-Ser-Tyr-Gln; SEQ ID NO:11), ENKISYQ (Glu-Asn-Lys-Ile-Ser-Tyr-Gln; SEQ ID NO:12), and ATKSKQH (Ala-Thr-Lys-Ser-Lys-Gln-His; SEQ ID NO:13). Determinations listed in the table were carried out with substrates at 0.2 mM concentration in the PSA assay buffer (50mM Tris/0.1M NaCl pH 7.8). The entry "UD" stands for "undetectable" and means that at most, 0.1 pmole of substrate cleavage per minute per 200 pmole protease took place. Asterisks represent experiments not performed. Abbreviations are as follows: PSA (prostate specific antigen), Chymo (chymotrypsin), Urokin (urokinase), TPA (tissue plasminogen activator), Thromb (thrombin), Kallik (human kallikrein, hK1).

Paragraph beginning at page 24, line 11, has been amended as follows:

The amino acid sequences expressed in one letter amino acid code represent the following amino acid sequences in the three letter amino acid code: EHSSKLQ (Glu-His-Ser-Ser-Lys-Leu-Gln; SEQ ID NO:10), HSSKLQ (His-Ser-Ser-Lys-Leu-Gln; SEQ ID NO:7), SKLQ (Ser-Lys-Leu-Gln; SEQ ID NO:1), and ATKSKQH [(](Ala-Thr-Lys-Ser-Lys-Gln-His; SEQ ID NO:13). Determinations listed in the table were carried out with substrates at 0.2 mM concentration in the PSA assay buffer (50mM Tris/0.1M NaCl pH 7.8).

Paragraph beginning at page 25, line 10, has been amended as follows:

The amino acid sequences expressed in one letter amino acid code represent the following amino acid sequences in the three letter amino acid code: EHSSKLQ (Glu-His-Ser-Ser-Lys-Leu-Gln; SEQ ID NO:10), [HSSKLQ (His-Ser-Ser-Lys-Leu-Gln), SKLQ (Ser-Lys-Leu-Gln),] QNKISYQ (Gln-Asn-Lys-Ile-Ser-Tyr-Gln; SEQ ID NO:11), ENKISYQ (Glu-Asn-Lys-Ile-Ser-Tyr-Gln; SEQ ID NO:12), and ATKSKQH [(](Ala-Thr-Lys-Ser-Lys-Gln-His; SEQ ID NO:13). Determinations listed in the table were carried out with substrates at 0.2 mM concentration in the PSA assay buffer (50mM Tris/0.1M NaCl pH 7.8). AMC is 7-amino-4-methylcoumarin. The human serum used was 100% for each assay. The entry "UD" stands for "undetectable", and means that not more than 0.01 picomole of substrate per minute was cleaved.

Paragraph beginning at page 25, line 20, has been amended as follows:

A family of peptide substrates based upon the EHSSKLQ (SEQ ID NO:10) sequence was assayed for activity for the intracellular proteases, and the results given in Table 4.

Paragraph beginning at page 25, line 32, has been amended as follows:

The amino acid sequences expressed in one letter amino acid code represent the following amino acid sequences in the three letter amino acid code: EHSSKLQ (Glu-His-Ser-Ser-Lys-Leu-Gln; SEQ ID NO:10), HSSKLQ (His-Ser-Ser-Lys-Leu-Gln; SEQ ID NO:7), and

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(Ser-Lys-Leu-Gln; SEQ ID NO:1). The shorter sequences are formed by deleting amino acids from the amino terminal side of the sequence. Determinations listed in the table were carried out with substrates at 0.2 mM concentration in the PSA assay buffer (50mM Tris/0.1M NaCl pH 7.8), except SKLQ (SEQ ID NO:1), KLQ and LQ, which were carried out in 1.4% acetonitrile/buffer and Q-AMC which was carried out in 0.2% formic acid/buffer, at pH 7.8. The entry "UD" stands for "undetectable" and means that at most, 0.1 pmole of substrate cleavage per minute per 200 pmole protease took place. Asterisks represent experiments not performed. Abbreviations are as follows: PSA (prostate specific antigen), Cath B, C, D (Cathepsins, B, C, D), Esterase (porcine liver esterase).

Paragraph beginning at page 40, line 12, has been amended as follows:

The Mu-HSSKLQ-AMC (SEQ ID NO:7) substrate was custom synthesized by Enzyme Systems Products (Dublin, CA) and characterized as described in Denmeade *et al.*, *Cancer Res.*, 57:4920-4926, (1997). Doxorubicin (Dox) prodrugs [Ac-His-Ser-Ser-Lys-Leu-Gln-Dox (HSSKLQ-Dox; SEQ ID NO:7) where Ac is acetyl] and [His-Ser-Ser-Lys-Leu-Gln-Leu-Dox (Mu-HSSKLQ-Leu-Dox; SEQ ID NO:14)where Mu is morpholinocarbonyl] were synthesized by coupling the primary amine of doxorubicin to the carboxyl group of the C-terminal amino acid. Purification of both compounds by HPLC yielded the trifluoroacetate salt (>98% purity). The peptide sequence was confirmed by amino acid analysis and molecular weights were confirmed by mass spectroscopy.

Paragraph beginning at page 40, line 27, has been amended as follows:

Table 6 shows the clonogenic survival of TSU-Pr1 cells following 48 hours of treatment with Mu-His-Ser-Ser-Lys-Leu-Gln-Leu-doxorubicin (SEQ ID NO:14) prodrug with and without 30 μg/ml enzymatically active PSA. Results for Mu-His-Ser-Ser-Lys-Leu-Gln-doxorubicin (SEQ ID NO:7) at 50 μM, were 122 colonies for treatment without PSA, and 110 colonies for treatment with 30 μg/ml enzymatically active PSA. Results are shown as averages (n = 5) with standard error of 2 to 7. Assays were done in triplicate.

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In the claims:

Claims 30 and 31 have been amended as follows:

30. (Amended) The composition of claim 19, wherein the peptide is His-Ser-Ser-Lys-Leu-Gln-Leu (SEQ ID NO:14).

31. (Amended) The composition of claim 19, wherein the therapeutic drug is a compound belonging to the group of thapsigargins which have been derivatized with a moiety containing a primary amino group, the peptide is His-Ser-Ser-Lys-Leu-Gln (SEQ ID NO:7), and the linker is selected from the group consisting of unsubstituted or alkyl-, aryl-, halo-, alkoxy-, alkkenyl-, amido-, or amino-substituted $CO\text{-}(CH{=}CH)_{n1}\text{-}(CH_2)_{n2}\text{-}Ar\text{-}NH_2$, $CO\text{-}(CH_2)_{n2}\text{-}(CH{=}CH)_{n1}\text{-}Ar\text{-}NH_2$, $CO\text{-}(CH_2)_{n2}\text{-}(CH{=}CH)_{n1}\text{-}CO\text{-}NH\text{-}Ar\text{-}NH_2$ and $CO\text{-}(CH{=}CH)_{n1}\text{-}(CH_2)_{n2}\text{-}CO\text{-}NH\text{-}Ar\text{-}NH_2$ wherein n1 and n2 are from 0 to 5, Ar is any substituted or unsubstituted aryl group, and attachment of $NH_2$ to Ar is in a ortho, meta or para position with respect to the remainder of the linker.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : John T. Isaacs et al.
Serial No. : 09/588,921
Filed : June 7, 2000
Title : TISSUE SPECIFIC PRODRUG

Art Unit : 1642
Examiner : S. Huff



**BOX SEQUENCE**
Commissioner for Patents
Washington, D.C. 20231

REQUEST UNDER 37 C.F.R. §1.821(e) TO TRANSFER COMPUTER READABLE FORM

The paper copy of the Sequence Listing in the above-referenced divisional/continuation application, is identical with the computer-readable copy of the Sequence Listing filed in U.S. Serial No. 09/081,707, filed May 5, 1998, from which this present application claims priority. In accordance with 37 C.F.R. §1.821(e), please use last-filed computer readable form filed in U.S. Serial No. 09/081,707, filed May 5, 1998, as the computer readable form for the instant application.

It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the instant application. A paper copy of the Sequence Listing is included herewith for the instant application.

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

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Signature

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Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: August 23, 2001

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